June 8, 1959

TO : Dr. A. W. Lindquist, Chief, Insects Affecting Man and Animals Research Branch

FROM : R. C. Bushland, Head, Insects Affecting Animals Section

SUBJECT: The Research Background for Screw-worm Eradication

At the planning conference in Sebring two weeks ago, Mr. Bruce told me that he already had arranged with you and Dr. Knipling for the Entomology Research Division to write two sections of a report on screw-worm eradication which would be submitted by the Animal Disease Eradication Division. I was to write a brief history of the research done for the current program, and Dr. Knipling was to write an outline of research needs for any future program in the Southwest and Mexico.

Mr. Bruce said that the deadline for the final report was June 30 so he asked me to prepare my section as soon as possible.

Enclosed are the original and two carbon copies of my draft of the section on research. Would you please make any editorial changes necessary and forward the section to Mr. Bruce without referring it to me for concurrence since I will be gone from Kerrville for the remainder of the month.

I am sending copies of the first draft and this covering letter to Mr. Bruce and Dr. Williams for their information only. Mr. Bruce will await the edited copy from your office.

cc: Mr. W. G. Bruce
Dr. D. L. Williams

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THE RESEARCH BACKGROUND FOR SCREW-WORM ERADICATION

by

R. C. Bushland
Head, Section of Insects Affecting Animals
Entomology Research Division
Agr. Res. Serv., U. S. D. A.
Kerrville, Texas

The first worker to observe that insects could be sterilized was G. A. Runner, an entomologist of the U. S. Department of Agriculture, who published in the Journal of Agricultural Research in 1916. He found that cigarette beetles infesting tobacco were either killed or sterilized by large doses of x-rays. No practical application was made of Runner's discovery and his work went largely unnoticed.

In 1927 H. J. Muller, a geneticist working at the University of Texas, published in Science that he could change the heredity of fruit flies by treating the males with x-rays. Muller's work attracted the attention of geneticists who were seeking means to induce mutations for studies in heredity. These geneticists found that they could not safely give more than a few hundred roentgens of irradiation to male fruit flies because higher doses caused such extensive chromosome changes in the sperm of irradiated males that their progeny could not live. These mutations that caused death were referred to in the genetics literature as "dominant lethal mutations." Many geneticists worked with various species of fruit flies, fungus gnats, and parasitic wasps and observed these effects from overdoses of x-rays or gamma rays

but no one seemed to consider any practical application for this phenomenon. Those specialists in heredity were more interested in having their organisms survive so that they could study the inheritance of changed characteristics. So the sterilizing effects of radiation were known among this group of specialists who used this knowledge largely to avoid overdosing their experimental insects but no one seemed to consider radiation as a possible toql for insect control.

In 1936 Roy Melvin and R. C. Bushland, working in the Dallas laboratory of the Entomology Research Division, developed a practical, inexpensive means for mass rearing of screw-worm flies in the laboratory, although in nature screw-worms can grow only in wounds in living warm-blooded animals. Those entomologists found that larvae could be grown in a laboratory incubator at body temperature on a mixture of ground lean meat, citrated beef blood and water with a little formaldehyde added to retard putrefaction. The only expensive ingredient in this formula was the ground meat,and each pound of meat furnished food for 500 larvae to complete their growth.

In 1938 E. F. Knipling, working with caged screw-worm flies at the Menard laboratory, noted that the females appeared to mate only one time. He proposed in conversations with his scientific associates that if a means for sterilizing laboratory-reared screw-worm males could be developed, that it might be practical to produce enough sterilized flies in the laboratory for distribution in nature to out-number the wild flies and thus have a means for eradicating the isolated population in the Southeastern States.

Screw-worms had not existed in the Southeast prior to 1933 when they were presumably introduced through shipment of infested cattle into Georgia. Efforts to control screw-worms in that area by good animal husbandry practices and treatment of wounds with benzol and pine tar oil had greatly reduced infestations during a control program in the years 1935-37. However, screw-worms could not be eradicated because enough breeding persisted in wild life and neglected domestic animals to maintain the infestation.

It was Dr. Knipling's idea that this residue of flies might be overwhelmed by sterilized insects. If sterilized males outnumbered native males and competed effectively for mates, the majority of the native females would make sterile matings and be unable to reproduce. By maintaining continuous releases of large numbers of sterile insects, each generation of native insects would be further outnumbered so that theoretically within a few generations the mathematical odds against a fertile mating would be so great that eradication would result.

When the Kerrville laboratory was established in 1947, Dr. Knipling directed that studies be made on the mating habits of screw-worm flies and that chemical sterilizing agents be tested. The experiments with chemical sterilizing agents were unsuccessful.

In 1950 Dr. Muller wrote an article in American Scientist in which he mentioned the sterilizing effects on insects of large doses of x-rays and gamma rays. A. W. Lindquist read this article and forwarded it to Dr. Knipling who ordered that the Kerrville laboratory begin investigations of the effects of radiation on screw-worms. R. C. Bushland and

D. E. Hopkins of the Kerrville laboratory first exposed screw-worms to x-rays using facilities at Brooke Army Hospital at Fort Sam Houston. They found that screw-worm pupae, after developing for five days at a constant temperature of 80° F., were susceptible to sterilization. A dose of 5000 r of x-rays sterilized both males and females. A dose of 7500 r completely prevented production of eggs in irradiated females. It reduced the sperm count by about half. Eggs fertilized by the irradiated sperm died without hatching; thence the males were called "sterile." The irradiated flies tolerated about three times the sterilizing dose. This provided an adequate margin of safety to avoid killing the insects in the sterilization procedure. The sterilized flies did not live quite as long as untreated insects but the sterilized males competed for mates with normal males in cage tests.

The Oak Ridge National Laboratory loaned the Kerrville laboratory a small cobalt 60 gamma ray source and trained Dr. Bushland in the safe handling of radioisotopes. Then the Kerrville workers experimented with gamma rays and confirmed the earlier work with x-rays. All the laboratory experiments at Kerrville with caged screw-worm flies indicated the practicality of controlling screw-worms in nature by outnumbering them with gamma ray-sterilized insects. This work was published by Bushland and Hopkins in the Journal of Economic Entomology in 1951 and 1953.

In 1952 a small research team from Kerrville was transferred to Florida to conduct practical field tests. Screw-worms were first reared and sterilized at Kerrville and shipped to Florida for release

in field experiments. Later in cooperation with Oak Ridge a gamma ray source especially made for sterilizing insects was installed at the Orlando laboratory. Field observations in Florida confirmed the Texas laboratory work. Sterilized screw-worms were released on Sanibel Island off the West Coast of Florida in numbers greater than existed in nature on that island. The natural population of screw-worms was greatly reduced but eradication could not be proved because the island was so close to the mainland that new flies migrated in to lay fertile egg masses.

The conclusive experiment was performed in 1954 when the Kerrville research team eradicated screw-worms from the island of Curacao. A. J. Graham, D. E. Hopkins, and F. H. Dudley reared and sterilized the flies at Orlando and shipped them by air freight to Curacao where they were released by A. H. Baumhover, W. D. New, and B. A. Bitter (a cooperating Dutch agricultural scientist). They found that when Curacao was treated twice weekly at the rate of 400 sterilized flies per square mile per week by distributing the flies in half-mile swaths from an airplane that the native population was outnumbered. During the first month of releases 69% of the egg masses laid by wild flies were infertile (indicating mating with the released males). After four weeks of such releases, when a new generation appeared, the number of wild flies was greatly reduced and the percentage of sterility had increased to about 90%. In another month only a few sterile egg masses could be collected, and the last sterile egg mass was collected after fourteen weeks of releases. Releases were continued for eight more weeks

to assure eradication. Screw-worms have not reappeared on Curacao. The details of this work were published in the Journal of Economic Entomology in 1955 by Baumhover et al.

For the Curacao work, 170,000 screw-worms had to be reared each week. To conduct an eradication campaign in Florida at the same average rate of release would require 50 million flies per week. This made it necessary to do many more experiments on mass culture methods and large-scale release operations in order to develop practical procedures for a Southeastern eradication program.

First of all, a new insectary was constructed with a capacity of one million larvae per week. We are indebted to E. J. Kendra and C. E. Cottrell, Jr., engineers, and Dale Jenkins, entomologist, Chemical Corps Engineering Command, Fort Detrick, Maryland, for their assistance in designing mass-rearing facilities.

For 20 years the rearing procedure of Melvin and Bushland had been used with little change. The maggots were reared in tubs or trays in cabinets or rooms heated to about 100° F. In cold weather ventilation was reduced to maintain temperatures, and the accumulation of foul odors and ammonia in the air of the building made working conditions difficult.

A. J. Graham and F. H. Dudley devised rearing vats heated from the bottom, and this equipment was refined and improved by C. N. Husman. This change in technique made it possible to rear the larvae in well-ventilated rooms, since the thermostatically equipped vats function independently of the room temperature. The new technique also more closely simulates the conditions in nature where the larvae grow in a

warm-blooded host and breathe cool air. This change to vat-rearing made it possible to double the capacity of the pilot plant, so that two million flies could be reared weekly in the same space.

The routine procedure was to induce oviposition by capturing gravid females in shell vials containing pieces of fresh lean meat and holding them for two hours at 90°-100° F. This practice became very laborious when thousands of females had to be egged for mass cultures. A. J. Graham and F. H. Dudley found that they could induce flies to oviposit merely by placing a heated attractant in a cage of flies where the room temperature was 80° F. A. H. Baumhover and Murray Brandstein joined in the effort on oviposition attractants and the group developed a standard technique for egging large cultures.

The juice that drains from thawing frozen horse meat or ground beef heart is heated for about two days at 98° F. The products of bacteria growing in this fluid are highly attractive to ovipositing flies. This meat juice is used to moisten fresh ground meat, which is placed in an electrically heated dish and inserted in a colony cage when the flies are seven days old. The air temperature is 80° and the oviposition medium is heated to 100°. The gravid females flock down upon this medium to feed and oviposit, and more than 90% of them lay their eggs within three or four hours.

All the laboratory and field work through the Curacao experiment was performed with a strain of flies that had been reared in Texas field laboratories since 1935 and had been inbred for the first ten years. After 1946 there were introductions of new genes by collecting egg masses

from ranch animals in the vicinity of Kerrville, rearing these new collections to the adult stage, and adding the flies to the laboratory breeding colony.

Since the Southeastern infestation had existed as an isolated population since 1933, it seemed that screw-worms might have adapted themselves to the subtropical and humid conditions of Florida. If so, the native males might be better fitted to compete in that environment than those of the Texas laboratory strain, which originated from a semi-arid region. Therefore, C. C. Skipper collected egg masses from natural screw-worm cases in Florida and southern Georgia, and A. H. Baumhover developed a laboratory strain from those collections. He selected for propagation only those flies that lived longest under laboratory conditions. Also, by confining individual males in cages with 25 females and rearing only those insects from the cages in which the most females were fertilized, he developed a laboratory strain in which the males mate about 17 times if provided with enough females.

The longevity and mating vigor of the Florida strain may be partly due to careful selection, but certainly much of the credit must be given to the improved techniques for rearing and maintaining laboratory cultures which were developed at Orlando during the period 1954 through 1957.

A. H. Baumhover and C. N. Husman worked on more efficient procedures for packaging and distributing sterilized flies. They adapted the standard one-pound frozen-food carton to provide a ventilated paper box with a capacity of 400 flies. Pupae are placed in the box after

irradiation, and the boxes are held under controlled temperature and humidity until the adults emerge. Then the flies are released from an airplane equipped with a motor-driven mechanism which opens and releases the cartons at any preset rate of delivery.

The 70-curie gamma ray source, which had been entirely satisfactory for sterilising flies released in previous experiments, did not have the capacity for mass sterilization. M. E. Jefferson, radiological safety officer of the Agricultural Research Service, and J. F. Mullins, of the Instrument Design Section at Beltsville, Maryland, with the cooperation of engineers of Brookhaven National Laboratory, designed and installed at Orlando a 600-curie source which can sterilize 18,000 pupae every seven minutes.

During the summer of 1957 these facilities were used in a large-scale field test in which the Animal Disease Eradication Division and the Florida Livestock Board also participated to determine the practical application of the sterilized fly technique for screw-worm control over a large area.

A 2,000-square-mile area in Florida in which a screw-worm outbreak was occurring, with flies more numerous than had ever been observed before on Curacao or in Florida, was treated with about 500 males per square mile per week for ten consecutive weeks. After the area was saturated with sterilized flies, hatching records on collected egg masses indicated that approximately 70% of the native females mated with sterilized males.

Eradication was not attained because this test area was not isolated from surrounding heavily infested territory and there was considerable migration of flies, as evidenced by the collection of sterile egg masses thirty miles away from the release area. However, the fact that 70% of the matings were sterile (a proportion that succeeded on Curacao) during outbreak conditions confirmed the competitive mating of sterilized males and showed that millions of flies could be reared, sterilized, and released in a continuing program. The experience gained in this test was invaluable in making plans for the eradication program now underway.

It should be pointed out that the research leading to the eradication program required seven years to complete. The work could have been done much more rapidly if more funds had been available. There was no special appropriation for screw-worm sterilization research for the two years of laboratory work at Kerrville which was accomplished with the generous cooperation of Army officials at Fort Sam Houston, who provided free x-ray services, and by the Biological Division of Oak Ridge National Laboratory who loaned the first cobalt-60 source free of charge and provided the larger source at nominal cost. For the Florida field work and the Curacao experiment there was a special appropriation of only $25,000 per year.

In addition to the research on screw-worm sterilization, the Kerrville laboratory made important progress on insecticide development as part of its regular program in screw-worm research for the immediate benefit of livestock producers in all parts of the country. All of

the insecticides now recommended for screw-worm treatment are developments of the Entomology Research Division. The first recommended treatment was benzol and pine tar oil, which originated in the Uvalde laboratory. Then came Smear 62 as a product of research at the Menard laboratory. The Kerrville laboratory developed Smear HQ-335. The screw-worm killing and wound protecting value of Bayer 21/199 (now sold as Co-Ral) were discovered at Kerrville. The specifications for Co-Ral sprays and dusts used in connection with field operations and quarantine procedures as part of the eradication effort are products of Kerrville research.